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First description of the female of *Cyrtodactylus dianxiensis* Liu & Rao, 2021, with extended diagnosis of this species (Squamata, Gekkonidae)

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Abstract

Cyrtodactylus dianxiensis Liu & Rao, 2021 was originally described based on only two adult male specimens from Tongbiguan Nature Reserve, Dehong Autonomous Prefecture, western Yunnan, China. So far, no information on the females of this species is available. During comprehensive herpetofaunal investigations in 2022, one female specimen of *C. dianxiensis* was collected from Tongbiguan Nature Reserve. The female specimen agrees well with the original description of *C. dianxiensis*, and also shows some slight differences in coloration. This study reported the female specimen of this species for the first time, and provided a description and photos of the female specimen; meanwhile, we extended the diagnosis of this species.

Key Words

bent-toed gecko, China, morphology, Yunnan

Introduction

Cyrtodactylus Gray, 1827 (bent-toed geckos) is the most speciose and ecologically diverse gekkonid genus, with more than 350 recognized species distributed from the Western Himalayas through southeast Asia to the Western Pacific (Wood et al. 2012; Uetz et al. 2024). Most species of *Cyrtodactylus* are karst-adapted or granite-adapted species (Grismer et al. 2020). With the in-depth investigation of karst areas, the underestimated biodiversity of *Cyrtodactylus* has been gradually revealed (Grismer et al. 2018; Liu et al. 2021, 2023).

So far, ten species of *Cyrtodactylus* have been recognized in China, and all are distributed in southwestern China, namely Yunnan Province and Tibet Autonomous Region. Four of them were recorded in Tibet: *C. tibetanus* (Boulenger, 1905), *C. cayuensis* Li, 2007, *C. zhaoermii* Shi & Zhao, 2010 and *C. kamengensis* Mirza, Bhosale, Thackeray, Phansalkar, Sawant, Gowande, Patel & Kamengensis, 2022. The other six were recorded in Yunnan: *C. dianxiensis* Liu & Rao, 2021, *C. gulinqingensis* Liu, Li, Hou, Orlov & Ananjeva, 2021, *C. hekouensis* Zhang, Liu, Bernstein, Wang & Yuan, 2021, *C. zhenkangensis* Liu & Rao, 2021, *C. menglianensis* Liu & Rao, 2022 and *C. caixitaoi* Liu, Rao, Hou, Wang & Ananjeva, 2023, among which, *C. dianxiensis* belong to the *C. khasiensis* species group, and the remaining belong to the *C. chauquangensis* species group.

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Cyrtodactylus dianxiensis is similar to *C. khasiensis* and previously the species have been confused with one another (Liu and Rao 2021; Wang et al. 2022). Liu and Rao (2021) describe the population of *C. khasiensis* distributed in Western Yunnan as a new species, *C. dianxiensis*. *Cyrtodactylus dianxiensis*, currently the only forest-dwelling *Cyrtodactylus* species recorded in Yunnan Province, China, was described based on one adult male holotype (KIZL2019044) from Tongbiguan Township, Yingjiang County, and one adult male paratype (KIZ2003170) from Longchuan County, both in Tongbiguan Nature Reserve, Dehong Autonomous Prefecture, western Yunnan, China (Liu and Rao 2021); no female information about this species was available. Identification verification of *C. khasiensis* voucher specimens deposited in museums could potentially yield additional specimens of *C. dianxiensis*. However, we have not yet been given the opportunity to examine the relevant specimens in other museums.

During a comprehensive herpetofaunal survey of Tongbiguan Nature Reserve in 2022, one female specimen of *Cyrtodactylus* was collected from Tongbiguan Nature Reserve, Xueli Village, Taiping Town, Yingjiang County, Dehong Prefecture, Yunnan Province, China (Fig. 1). Morphological and molecular phylogenetic analyses revealed that the specimen is *C. dianxiensis*. Here, we provide morphological data for the newly collected female specimen of *C. dianxiensis*, and extend the diagnosis of this species.

Materials and methods

Sampling

The specimen was sampled by hand at night. Sex was further determined based on whether the ventral side of the tail base is swollen. After euthanasia, liver tissues were taken and preserved in 95% alcohol. The specimen was then directly preserved in 75% ethanol without formalin fixation. The voucher specimen was deposited in Kunming Natural History Museum of Zoology, Kunming Institute of Zoology, Chinese Academy of Sciences (KIZ).

Morphological analyses

The adult female specimen preserved in 75% ethanol was measured with digital calipers to the nearest 0.1 mm. Morphological terminology followed Liu and Rao (2021). Measurements included the following: snout-vent length (SVL), from tip of snout to anterior margin of cloaca; trunk length (TRL), axilla to groin distance; body width (BW), maximum width of body; tail length (TL), from posterior margin of cloaca to tip of tail; tail width (TW), maximum width of tail; head length (HL), from tip of snout to posterior margin of jaw; head width (HW), maximum width of head; head

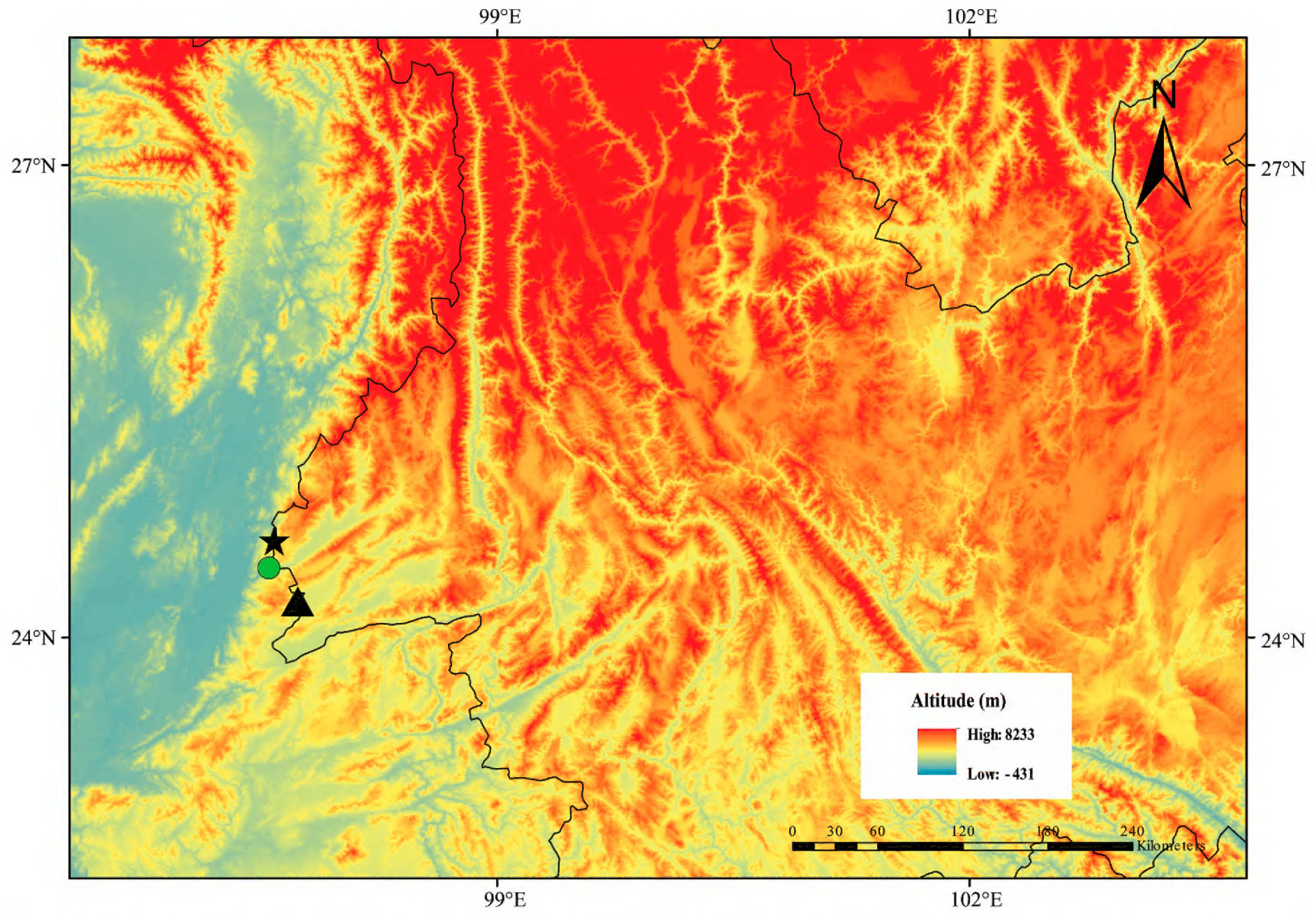

Figure 1. Sampling sites of *Cyrtodactylus dianxiensis*: solid black star, the locality of the holotype; solid black triangle, the locality of the paratype; solid green circle, the locality of the newly collected female specimen.

height (HH), from occiput to underside of jaws; forearm length (FL), from the base of the palm to the elbow; crus length (CL), from the base of heel to the knee; eye diameter (ED), greatest diameter of eye; nostril to eye distance (NE), from nostril to anterior corner of eye orbit; snout to eye distance (SE), from tip of snout to anterior corner of eye orbit; orbit to ear distance (EE), from posterior corner of eye orbit to anterior margin of ear opening; ear length (EL), greatest diameter of ear; internarial distance (IN), measured between inner borders of nostrils; interorbital distance (IO), measured across narrowest point of frontal bone. The counting of the following characteristics also followed Liu and Rao (2021): dorsal tubercle rows (DTR); ventral scale rows (MVSR); paravertebral tubercles (PVT); supralabials (SL); infralabials (IL); precloacal pores (PcP); precloacofemoral pores (PcFP); femoral pores on each thigh (FP); subdigital lamellae under the fourth finger (LF4) and under the fourth toe (LT4).

DNA extraction, PCR amplification, and sequencing

Total genomic DNA was extracted from tissue samples preserved in 95% ethanol. The tissue sample was then digested using proteinase K, and subsequently purified using DNeasy Tissue Kit (QIAGEN). A fragment of NADH Dehydrogenase subunit 2 (ND2) was amplified and sequenced using the primers L4437b and H5934 (Macey et al. 1997). The experiment protocols are the same as Liu and Rao (2021). New sequence was assembled and edited using SEQMAN in Lasergene 7.1 (DNASTAR Inc., Madison, WI, USA).

Molecular analysis

Phylogenetic relationships within the *C. khasiensis* species group were inferred from ND2. The homologous sequences of the *C. khasiensis* species group, and the outgroups species *C. slowinskii*, were downloaded from GenBank (Clark et al. 2016) (Table 1). Sequences were aligned using MUSCLE 3.6 (Edgar 2004), then checked by eye for accuracy and trimmed to minimize missing characters in MEGA 6.0.6 (Tamura et al. 2013).

Phylogenetic reconstruction was conducted using Bayesian inference (BI) and maximum likelihood (ML) methods based on the ND2 gene. The best-fit substitution model was selected under the Bayesian Information Criterion by the program MODELFINDER (Kalyaanamoorthy et al. 2017) implemented in IQ-TREE 1.6.12 (Nguyen et al. 2015). Bayesian inference analyses were performed in MRBAYES 3.2.7 (Ronquist et al. 2012) based on the model GTR+F+I+G4. Two independent runs were initiated, each with four simultaneous Markov Chain Monte Carlo (MCMC) chains for one million generations and sampled every 100 generations with a burn-in of 25%. The convergence was examined with an average standard deviation of split frequencies less than 0.01 and effective sample size (ESS) values greater than 200 in TRACER 1.5 (Rambaut and Drummond 2009). Maximum likelihood analyses were conducted using IQ-TREE 1.6.12 (Nguyen et al. 2015), based on the model TIM+F+I+G4. Nodal support was estimated by 1,000 bootstrap replicates using the ultrafast bootstrap feature. Pairwise divergences were calculated using uncorrected *p*-distances implemented in MEGA 6.0.6 (Tamura et al. 2013).

Table 1. Locality, voucher ID, and GenBank accession (ND2) for all samples used in this study. * denotes the holotype of *Cyrtodactylus dianxiensis*.

Species	Locality	Voucher	Accession
Cyrtodactylus aaronbaueri	Mizoram, India	MZMU 2015	MW596520
Cyrtodactylus agarwali	Meghalaya, India	MZMU 2158	MW596515
Cyrtodactylus arunachalensis	Arunachal Pradesh, India	BNHS 2777	MT341522
Cyrtodactylus aunglini	Mandalay, Myanmar	LSUHC 13948	MH764589
Cyrtodactylus ayeyarwadyensis	Ayeyarwady, Myanmar	CAS 212459	JX440526
Cyrtodactylus bapme	Meghalaya, India	BNHS 2756	MW367435
Cyrtodactylus bengkhuaiai	Mizoram, India	MZMU 1985	MW596516
Cyrtodactylus chrysopylos	Shan State, Myanmar	LSUHC 13937	MH764604
Cyrtodactylus dianxiensis	Yunnan, China	KIZ059201	MW971927
Cyrtodactylus dianxiensis	Yunnan, China	KIZL2019044*	MW971926
Cyrtodactylus dianxiensis	Yunnan, China	KIZ2022159	PP394340
Cyrtodactylus exercitus	Meghalaya, India	MZMU 2545	OK247679
Cyrtodactylus gansi	Chin State, Myanmar	CAS 222412	JX440537
Cyrtodactylus guwahatiensis	Assam, India	BNHS 2146	KM255194
Cyrtodactylus jaintiaensis	Meghalaya, India	BNHS 2248	KM255195
Cyrtodactylus karsticola	Meghalaya, India	MZMU 2156	MW596513
Cyrtodactylus kazirangaensis	Assam, India	BNHS 2147	KM255170
Cyrtodactylus khasiensis	Meghalaya, India	BNHS 2249	KM255188
Cyrtodactylus lungleiensis	Mizoram, India	MZMU 2428	MZ645742
Cyrtodactylus montanus	Tripura, India	BNHS 2231	KM255200
Cyrtodactylus mombergi	Kachin State, Myanmar	LSUHC 14734	MN059875
Cyrtodactylus nagalandensis	Nagaland, India	BNHS 2253	KM255199
Cyrtodactylus namtiram	Manipur, India	BNHS 2751	MW367433
Cyrtodactylus ngopensis	Mizoram, India	MZMU 2360	OM912605
Cyrtodactylus septentrionalis	Assam, India	BNHS 1989	MH971164
Cyrtodactylus siahaensis	Mizoram, India	MZMU 2445	OK247677
Cyrtodactylus tripuraensis	Tripura, India	BNHS 2238	KM255183
Cyrtodactylus urbanus	Assam, India	VR/ERS/ZSI/688	MN911174
Cyrtodactylus vairengtensis	Mizoram, India	MZMU 2903	OP874800
Cyrtodactylus slowinskii	Sagaing, Myanmar	CAS 210205	JX440559

Results

Bayesian inference and ML trees showed consistent topology. The newly collected female specimen clustered with the specimens (including the holotype) of *C. dianxiensis* with strong support by both BI and ML (BI/ML=1/100, Fig. 2). The genetic distance (uncorrected *p*-distance) between the newly collected female specimen and the specimens (including the holotype) of *C. dianxiensis* was only 0.9% (Table 2).

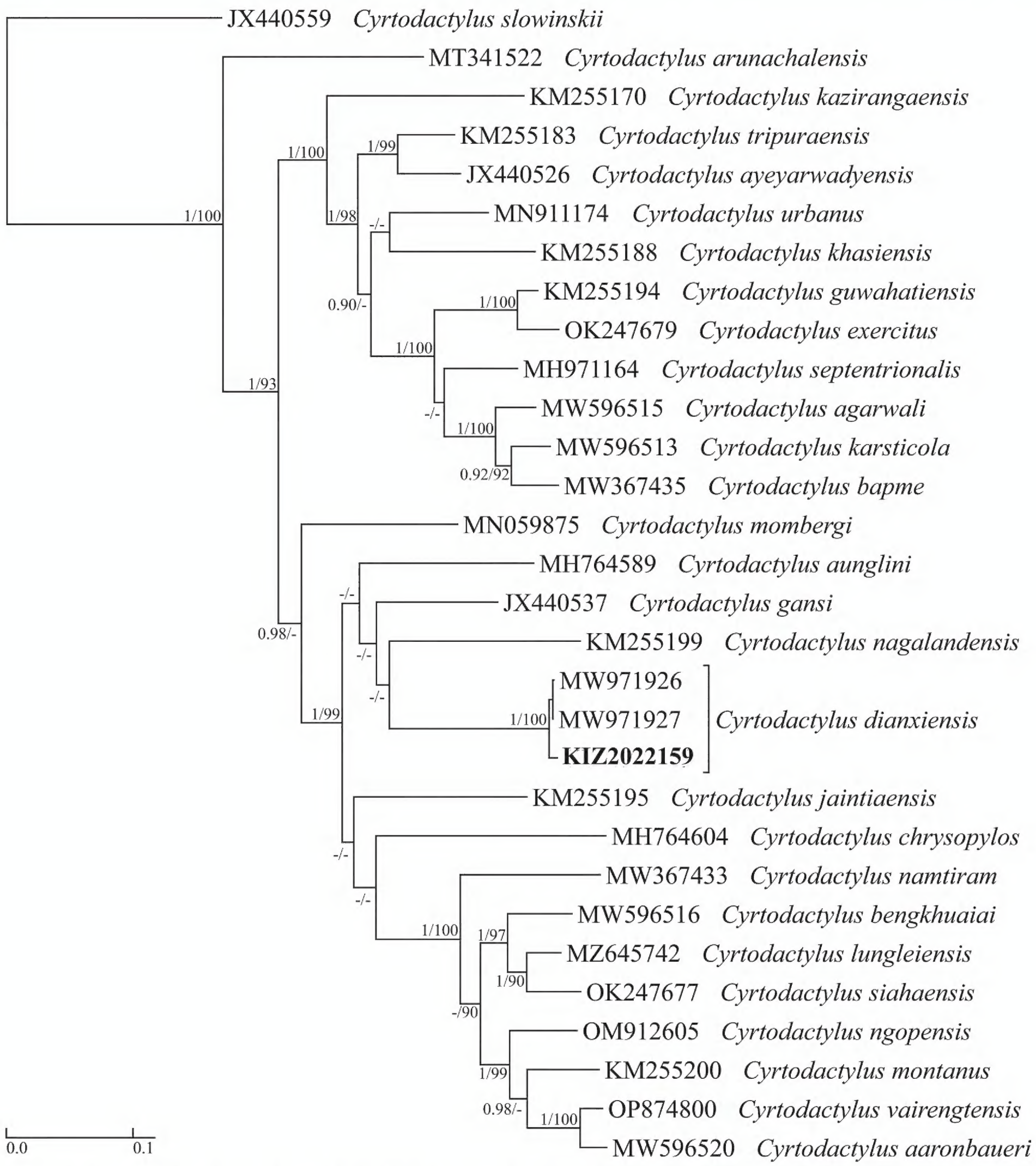

Figure 2. Bayesian phylogram of the *Cyrtodactylus khasiensis* species group inferred from ND2 sequences. Numbers before slashes indicate Bayesian posterior probabilities and numbers after slashes indicate the ML ultrafast bootstrap. Values below 0.90/90 are not shown.

Cyrtodactylus dianxiensis Liu & Rao, 2021

Figs 3–4, Table 3

Specimen examined. KIZ2022159, adult female, collected on 13 September 2022 by Shuo Liu from Tongbiguan Nature Reserve, Xueli Village, Taiping Town, Yingjiang County, Dehong Prefecture, Yunnan Province, China (24°26'35"N, 97°33'1"E; at an elevation of 380 m) (Fig. 1).

Description of the female specimen. SVL 75.0 mm; head relatively large (HL/SVL 0.26, HW/HL 0.68), depressed (HH/HL 0.45), distinct from neck; loreal and interorbital region concave, canthus rostralis slightly swollen; snout moderately long (SE/HL 0.40); eye large (ED/HL 0.24); pupil vertical with crenulated margins; ear opening oval, obliquely oriented (EL/HL 0.08); rostral with mid-rostral suture dorsally; two large supranasals, separated by one small internasal; dorsal head scales heterogeneous,

Table 2. Uncorrected pairwise divergence (%) between *Cyrtodactylus khasiensis* species group members based on ND2 sequences.

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
1	*C. aaronbaueri*																											
2	*C. agarwali*	24.3																										
3	*C. arunachalensis*	23.4	20.6																									
4	*C. aunglini*	19.6	20.8	24.2																								
5	*C. ayeyarwadyensis*	21.9	14.8	20.2	18.2																							
6	*C. bapme*	22.9	7.2	20.8	20.7	14.2																						
7	*C. bengkhuaiai*	12.0	23.6	20.9	18.3	19.9	21.9																					
8	*C. chrysopylos*	21.7	21.4	23.1	18.5	18.9	21.9	19.2																				
9	*C. dianxiensis*	21.2	20.3	21.9	16.6	18.8	20.4	21.0	18.8																			
10	KIZ2022159	21.0	20.6	21.8	19.8	21.7	20.7	21.0	22.0	0.9																		
11	*C. exercitus*	22.5	11.8	18.4	21.7	16.2	13.6	22.5	21.9	21.3	21.4																	
12	*C. gansi*	19.2	21.0	22.0	15.8	16.9	20.9	18.2	17.6	15.5	18.4	21.1																
13	*C. guwahatiensis*	22.6	12.5	20.3	21.6	15.4	13.6	22.1	22.9	22.4	22.7	4.5	21.1															
14	*C. jaintiaensis*	19.2	23.1	22.9	19.1	21.8	22.5	17.3	22.0	19.1	19.3	21.4	19.0	22.4														
15	*C. karsticola*	22.9	6.4	19.1	19.2	12.6	6.7	23.8	21.1	20.0	20.1	13.4	21.1	13.1	22.0													
16	*C. kazirangaensis*	24.7	20.0	21.5	22.5	16.7	19.6	22.9	23.7	21.0	21.3	18.3	21.7	19.8	23.0	17.1												
17	*C. khasiensis*	22.7	17.2	21.0	21.5	15.6	16.9	19.5	22.1	21.9	22.6	17.0	20.0	16.9	21.5	15.2	18.5											
18	*C. lungleiensis*	12.1	21.6	19.9	18.3	19.2	20.7	7.2	20.4	20.3	19.7	20.2	18.5	21.1	18.0	22.0	21.8	18.8										
19	*C. mombergi*	20.0	20.4	20.4	17.4	17.3	19.9	20.4	19.2	17.2	20.3	20.4	15.9	21.8	19.7	19.6	21.0	20.3	18.4									
20	*C. montanus*	10.3	22.9	22.6	19.6	20.0	20.5	12.1	21.5	19.5	20.1	20.0	19.6	21.9	18.8	22.0	23.3	21.7	10.9	19.6								
21	*C. nagalandensis*	21.5	24.8	22.3	19.8	21.7	23.8	19.2	22.7	19.0	19.4	22.6	18.0	23.6	19.0	22.0	23.1	22.0	19.1	20.6	19.6							
22	*C. namtiram*	15.7	22.6	22.5	20.4	20.9	22.3	14.2	20.7	21.9	22.0	23.3	20.3	23.3	20.0	24.1	23.7	21.9	13.9	19.5	15.4	19.4						
23	*C. ngopensis*	9.9	23.6	22.0	18.3	20.7	21.1	11.2	20.3	20.2	19.9	21.0	18.4	21.6	17.8	22.7	24.2	22.2	11.5	19.6	10.5	19.7	14.8					
24	*C. septentrionalis*	21.2	11.1	20.6	20.8	14.6	11.6	20.5	21.7	20.3	21.0	10.9	19.8	11.3	21.4	10.7	18.7	15.7	19.1	20.6	19.6	23.6	21.5	20.1				
25	*C. siahaensis*	11.4	22.9	20.0	18.7	20.1	20.9	8.6	20.8	19.3	19.1	21.8	18.9	21.1	17.5	23.4	22.5	19.4	6.1	19.1	11.0	17.2	14.2	10.3	19.3			
26	*C. tripuraensis*	21.8	13.2	18.4	18.8	7.5	12.3	18.4	19.6	18.9	19.8	14.7	16.7	12.9	18.6	13.1	14.5	12.5	19.1	17.1	18.2	19.0	21.1	18.8	11.5	18.6		
27	*C. urbanus*	23.8	15.6	20.8	21.3	13.7	15.1	21.5	21.5	20.8	21.1	14.9	20.0	15.2	21.8	14.3	17.3	14.2	20.1	20.5	21.8	22.9	21.6	21.4	13.4	20.1	11.5	
28	*C. vairengtensis*	4.0	24.4	24.2	18.9	21.1	22.2	11.2	21.1	20.8	20.5	21.9	18.5	22.2	18.8	22.3	24.5	22.8	11.7	20.4	10.2	20.1	15.9	10.3	20.5	10.6	20.0	22.3

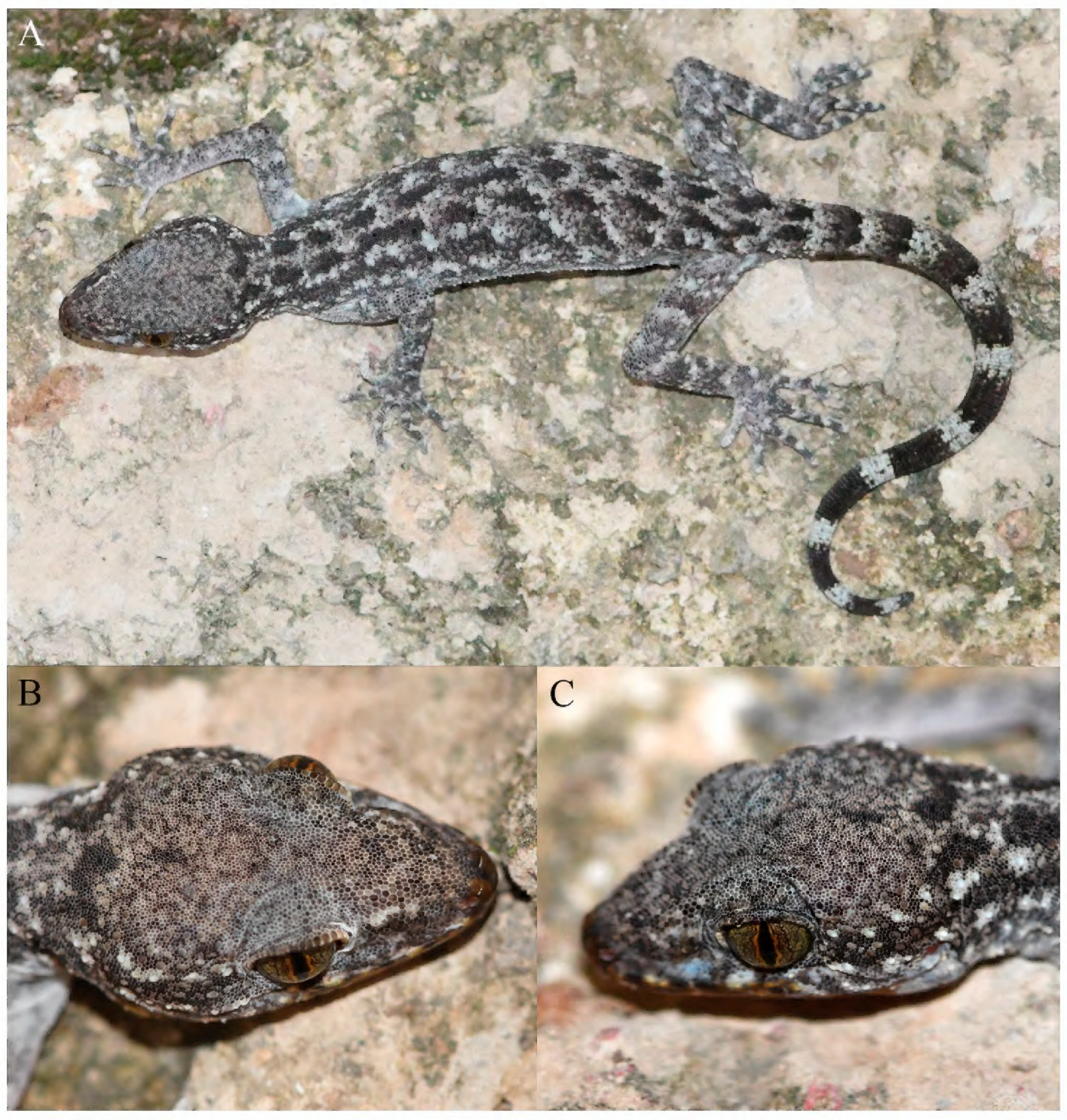

Figure 3. The female specimen (KIZ2022159) of *Cyrtodactylus dianxiensis* in life. **A.** Dorsal view; **B.** Close-up dorsal view of the head; **C.** Close-up dorsolateral view of the head.

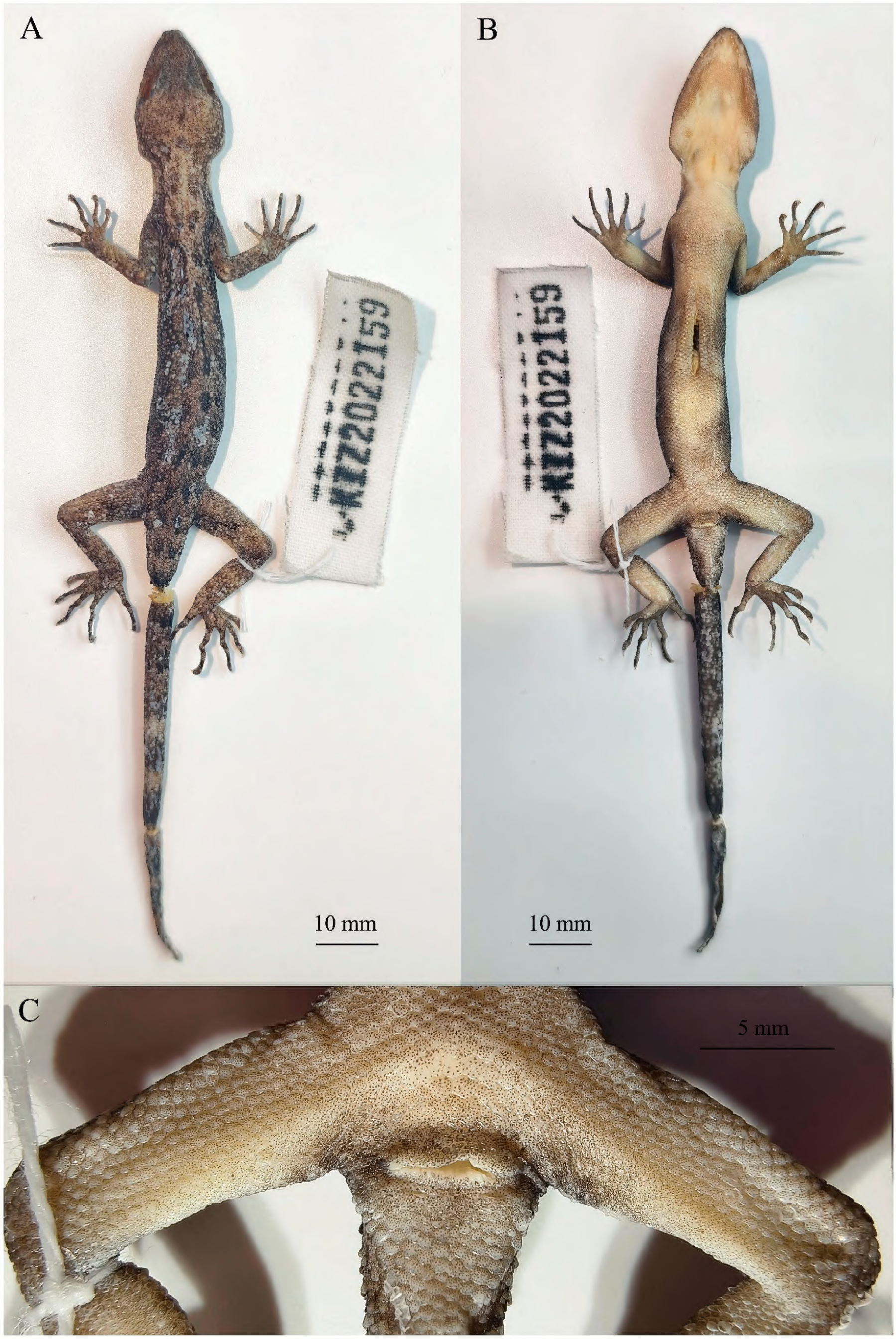

Figure 4. The female specimen (KIZ2022159) of *Cyrtodactylus dianxiensis* in preservative. **A.** Dorsal view; **B.** Ventral view; **C.** Close-up view of the precloacal and femoral regions.

Table 3. Measurements (mm) and meristic data for the female specimen of *Cyrtodactylus dianxiensis*. See Materials and methods for abbreviations.

	KIZ2022159		KIZ2022159
SVL	75.0	EE	5.9
TRL	33.8	EL	1.6
BW	11.6	IN	2.5
TL	73.7	IO	2.6
TW	6.8	FP	0
HL	19.3	PcP	0
HW	13.2	MVSR	35
HH	8.6	PVT	32
FL	11.5	DTR	17
CL	13.3	SL (R/L)	9(7)/10(7)
ED	4.6	IL (R/L)	9/8
NE	6.1	LF4	17/16
SE	7.8	LT4	20/20

rounded, granules; mental triangular with a pair of enlarged postmentals followed by some gradually decreasing chin-shields; scales on other region of the ventral head almost homogeneous, small, rounded, granules; supralabials seven to midorbital position on both sides, nine to angle of the jaw on right side and ten to angle of the jaw on the left side; infralabials nine on right side and seven on left side.

Body slender (TRL/SVL 0.45); dorsal scales heterogeneous, primarily small rounded granules, intermixed with irregularly arranged large circular tubercles, tubercles on nape and occipital region smaller than those on dorsum; ventrolateral folds present; ventral scales larger than dorsal; enlarged femoral scales and femoral pores absent; precloacal scales enlarged, precloacal pores absent; no precloacal groove; cloacal spurs indistinct.

Limbs slender (FL/SVL 0.15, CL/SVL 0.18); digits strongly inflected at each joint, all bearing robust, recurved claws; relative length of digits: I<II<V<III<IV (manus) and I<II<III<V<IV (pes); scales on dorsal forelimbs heterogeneous, granules, with small tubercles interspersed, scales on dorsal hind limbs heterogeneous, granular, with large conical tubercles interspersed; ventral scales of limbs almost homogeneous, granular, smaller than those on ventral body.

Original tail broken but exists, subequal to body length (TL/SVL 0.98); dorsal tail scales heterogeneous, with small tubercles interspersed; two rows of subcaudal scales slightly enlarged.

Coloration in life. Dorsal surface of head almost uniform brownish gray; upper lip brownish gray with many white spots, an indistinct black postorbital streak that extends backwards above ear opening on each side; a disconnected W-shaped black stripe on the occiput; dorsal surface of body brownish gray with many short black and white streaks roughly forming longitudinal rows; dorsal surface of limbs gray with indistinct white bands and spots; dorsal surface of tail grayish black with ten white bands; ventral surfaces of head, body, and limbs white, ventral surface of tail checkered with brownish gray and white; iris bronze with dark reticulations, edge of pupil orange red.

Extended diagnosis. Body size moderate (SVL 73.8–79.9 mm in adults); 9–12 supralabials; 8–11 infralabials; 17–19 longitudinal rows of rounded, conical dorsal tubercles; 31–32 paravertebral tubercles; 35–41 ventral scales between ventrolateral folds; no precloacal groove; femoral scales not enlarged, no femoral pores; 7–8 precloacals in males, no precloacal in female; 16–17 total subdigital lamellae beneath finger IV, 19–20 total subdigital lamellae beneath toe IV; subcaudal scales not transversely enlarged or two median rows slightly enlarged. Dorsum with light and dark blotches roughly forming longitudinal markings; a W-shaped black stripe present on occipital region; tail with 8–10 alternating dark and light bands; iris blueish gray or bronze with orange red edge.

Discussion

The female specimen agrees well with the original description (Liu and Rao 2021) of *C. dianxiensis* in measurements and meristic counts, however, there are some slight differences in coloration between them. The female specimen has a relatively lighter body color; the dorsal head of the holotype of *C. dianxiensis* is mixed with brown and grayish white, while it is almost uniform brownish gray in the female specimen; the iris of the holotype is blueish gray with no orange red edge, while the iris is bronze with orange red edge in the female specimen. Therefore, in this species, the coloration of dorsal head is not always mottled, but may also be uniform; in addition, the iris of this species has two color types, blueish gray and bronze with orange red edge.

The holotype of *C. dianxiensis* was collected at an altitude of 1170 m and the paratype was collected at an altitude of 1200 m (Liu and Rao 2021). However, the female specimen was collected at a much lower altitude. This record extends the lowest elevational distribution of this species to 380 m.

Cyrtodactylus dianxiensis was previously known based on only two adult males and one juvenile (Liu and Rao 2021). This study reported the female specimen of this species for the first time, and provided a detailed description and photos of the female of this species. However, we have only collected one female specimen of this species, and it is unknown whether some differences between male and female are permanent. In addition, the range and population status of this species will need to be determined by collecting more specimens in the future.

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